6. Jiang, Y.L., Stivers, J.T., and Song, F. (2002). Biochemistry *41*, 11248–11254.

7. Beuck, C., Singh, I., Bhattacharya, A., Hecker, W., Parmar, V.S., Seitz, O., and Weinhold, E. (2003). Angew. Chem. Int. Ed. Engl. *42*, 3958–3960.

8. Groebke, K., Hunziker, J., Fraser, W., Peng, L., Diederichsen, U., Zimmermann, K., Holzner, A., Leumann, C., and Eschenmoser, A. (1998). Helv. Chim. Acta *81*, 375–474.

9. Hoffmann, M.F.H., Bruckner, A.M., Hupp, T., Engels, B., and Diederichsen, U. (2000). Helv. Chim. Acta *83*, 2580–2593.

10. Liu, H., Gao, J., Lynch, S.R., Saito, Y.D., Maynard, L., and Kool, E.T. (2003). Science *302*, 868–871.

11. Gao, J., Liu, H., and Kool, E.T. (2005). Angew. Chem. Int. Ed. Engl. *44*, 3118–3122.

12. Orgel, L.E. (1968). J. Mol. Biol. *38*, 381–393.

13. Orgel, L.E. (1986). Orig. Life Evol. Biosph. *17*, 27–34.

14. Wächtershäuser, G. (1988). Proc. Natl. Acad. Sci. USA *85*, 1134–1135.

Exploiting Green Treasures

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In this issue of *Chemistry & Biology*, Ishida and colleagues [1] report on the characterization of new aeruginoside metabolites and the respective NRPS gene cluster in the cyanobacterium *Planktothrix agardhii*.

Cyanobacteria are among the most talented, culturable micro-organisms from which novel, structurally diverse, biochemically active natural products have been isolated. Many of the compounds are small nontoxic or toxic peptides with unusual amino acids, polyketides, or hybrids of different biosynthetic pathways [2, 3].

Cyanobacteria are structurally diverse, geographically widespread in freshwater, marine, and terrestrial habitats, and some genera are nitrogen fixing and are therefore of great importance for the natural balance of the ecology. Due to the production of highly active hepatotoxins and neurotoxins, increasingly common cyanobacterial water blooms are of serious concern in freshwater reservoirs or lakes [2, 3, 4]. Investigating these toxins and other bioproducts with a focus on cancer drug discovery and chemical ecology is a promising research field where culturable cyanobacteria play an outstanding role. Diverse metabolites with relevant pharmaceutical activities have been isolated from cyanobacteria. However, the true relevance of these highly active agents for the producer strains remains elusive at present.

In this issue of *Chemistry & Biology* [1], two analogs of cyanobacterial aeruginosides, produced by the genus *Oscillatoria* (syn. *Planktothrix*), are reported. More than 200 compounds originate from different species of the order *Oscillatoriales* [2]. In the last decade, the toxic *Planktothrix agardhii* received considerable attention, and was recognized as a prolific source of novel metabolites [5]. Several compounds from different chemical classes are currently known. These include the first aeruginosins (205A and 205B) reported from *P. agardhii* that were highlighted as glycopeptides inhibiting serine proteases [6], several variants of the hepatotoxin complex microcystin isolated from a single strain [7], and the multicyclic microviridins that are the largest known cyanobacterial oligopeptides with characteristic ester and secondary amino bonds [3]. However, the full spectrum of chemical compounds produced by different isolates of the *P. agardhii* species has not been cataloged. Perhaps more interestingly, the whole metabolite pattern of a single strain as a distinct cocktail of bioactive agents has not been investigated in detail with respect to its ecological and physiological relevance; although, Fujii and coworkers [5] gave some initial insights.

The approach taken by Ishida and colleagues [1] is a sophisticated application of the general knowledge about the organization of nonribosomal peptide synthetase (NRPS) biosynthetic gene clusters in cyanobacteria to probe a yet unexplored part of the metabolite pattern of *P. agardhii* strain CYA 126/8. The method used elegantly turns around a classic concept in natural product research. Typically, a biosynthetic gene cluster is identified long after the respective metabolite is identified and fully characterized in a chemical-pharmaceutical screening program [8]. In the new report, a NRPS gene sequence was identified by a degenerate PCR approach in *Planktothrix* and used for insertional mutagenesis with a chloramphenicol resistence cassette to give evidence for the involvement of the genes within the cluster in putative peptide biosynthesis. Importantly, parallel cultivation of wild-type cells and mutant cells called attention to previously unknown metabolites. Subsequent careful chemical analysis identified two new

aeruginosides, 126A and 126B, glycosylated peptides that consist of an N-terminal aryl lactic acid, D-leucine, the characteristic Choi moiety and a C-terminal arginine analog (Aeap). Additionally, one strong point of the approach [1] is the independence from pharmaceutically relevant bioactivities as the sole guidance for metabolite screening since 126A and 126B lack striking activity in standard tests. However, their functions need to be examined as they might be of importance for physiological and ecological processes in the producer strain, or for activity screens other than those normally used in pharmaceutical test systems.

An interesting question is whether the concept used by Ishida and colleagues [1] can lead to the discovery of additional peptide families encoded in the genome of this individual cyanobacterial strain. It would be of great interest to add yet unknown structures to the metabolite pattern of *P. agardhii*, even if they are restricted to NRPS-derived products with this strategy. Finding a so-called "silent biosynthetic gene cluster," one not related to obvious metabolite production under lab conditions, would be an advanced challenge for both biologists and chemists, and could mirror the organism's full biosynthetic potential. Other screening approaches might add to the picture of *P. agardhii* as an extraordinarily talented cyanobacterial producer strain [5].

Beyond understanding how the metabolites are made, there is direct, compound driven interest (potential pharmaceutical or cosmeceutical) in microbial, here cyanobacterial, metabolism. The diversity of chemical reactions performed with biochemical (i.e., enzymatic) means has attracted increasing interest from the chemical industry. Considering the growing importance of establishing "green" sustainable production processes, more and more chemical processes are being switched to biotechnological routes [9]. Moreover, "green" products will play an important role per se in the rapidly expanding field of dietary supplements to improve "beauty and health" by disease prevention, an emerging sector filling the gap between nutrition and classical pharmaceutical therapies [10].

The goal of industrial or white biotechnology is to provide the technologies (biological routes and systems) and discoveries to develop suitable pathways and biocatalysts in "green" production processes. Studies on biosynthetic routes to compounds featuring rare moieties (e.g., the Choi moiety described in this issue [1]) or special reaction types and tailoring activities have the potential to identify enzymes of interest. Enzymes capable of Michael addition (like AerE [1]), Friedel-Crafts-type electrophilic substitutions [11], or halogenases and prenyl transferases [12, 13, 14] provide excellent insights into the workings and capabilities of biological processes and importantly provide tools for biotechnological applications.

The concept of microbial diversity has dramatically expanded in recent years. However, according to Amman and coworkers [15] only a minority of micro-organisms living in any given habitat are cultivable, possibly due to complex interspecies associations in biofilms or symbiosis. In fact, studies by Torsvik and coworkers [16] and especially Venter and coworkers [17] defined the enormous dimensions of biological (i.e., molecular) diversity as a nearly inexhaustible resource of genes and pathways for biotechnological/industrial applications. Currently only metagenomics has the potential to make this entire resource available since this technology comprises the application of the genomics methods to the complete genomes of all micro-organisms living in a defined habitat. On the other hand, it is initially important to explore in detail the biosynthetic and metabolic treasures of particular phyla of microbial life. Studies such as the one in this issue [1] give detailed functional insights into the biosynthetic capabilities and hence enzymes or bioactive compounds are available for industrial driven investigations.

Two goals, the urgent need for therapeutic agents and the interest in understanding/using biological systems, are inherently connected and currently guide molecular cyanobacterial research. For example, it remains elusive whether a regulatory network of the different metabolites exists. The authors' research on aeruginosides builds an ideal basis for such investigations either in the lab or in a natural habitat [1, 18]. Great interest in this area comes particularly from the rise of pathogenic bacteria in clinical settings. There, the aim is to successfully defeat the microorganisms. In a related example, we are keen to understand the functional role of secondary metabolites, for example microcystin, in planktonic cyanobacterial communities where they might comprise grazing protection or cell-cell signaling mechanisms. Future research might take the exciting direction of considering that the comprehensive group of structural variants and relative amounts of compounds produced by a single bacterial species is important as a very distinct cocktail of biological interacting chemicals with ecological advantages and consequences for the producer.

REFERENCES

1. Ishida, K., Christiansen, G., Yoshida, W.Y., Kurmayer, R., Welker, M., Valls, N., Bonjoch, J., Hertweck, C., Börner, T., Hemscheidt, T., et al. (2007). Chem. Biol. *14*, this issue, 565–576.

2. Burja, A.M., Banaigs, B., Abou-Mansour, E., Burgess, J.G., and Wright, P.C. (2001). Tetrahedron *57*, 9347–9377.

3. Welker, M., and von Döhren, H. (2006). FEMS Microbiol. Rev. *30*, 530–563.

4. Chorus, I. (2001). Cyanotoxins - Occurrence, Causes, Consequences (Berlin, Heidelberg, New York: Springer-Verlag), 5–101.

5. Fujii, K., Sivonen, K., Naganawa, E., and Harada, K. (2000). Tetrahedron *56*, 725–733.

6. Shin, H.J., Matsuda, H., Murakami, M., and Yamaguchi, K. (1997). J. Org. Chem. *62*, 1810–1813.

7. Sano, T., and Kaya, K. (1998). Tetrahedron *54*, 463–470.

8. Christiansen, G., Fastner, J., Erhard, M., Börner, T., and Dittmann, E. (2003). J. Bacteriol. *185*, 564–572.

9. Zinke, H. (2006). Biotechnol. J. *1*, 717–718.

10. Mueller-Kuhrt, L. (2007). Expert Opin. Drug Des. Discov. *2*, 305–311.

11. Retey, J. (1996). Naturwissenschaften *83*, 439–447.

12. Chang, Z.X., Flatt, P.M., Gerwick, W.H., Nguyen, V.A., Willis, C.L., and Sherman, D.H. (2002). Gene *296*, 235–247.

13. Rouhiainen, L., Paulin, L., Suomalainen, S., Hyytiainen, H., Buikema, W., Haselkorn, R., and Sivonen, K. (2000). Mol. Microbiol. *37*, 156–167.

14. Edwards, D.J., and Gerwick, W.H. (2004). J. Am. Chem. Soc. *126*, 11432–11433.

15. Amman, R.I., Ludwig, W., and Schleifer, K.H. (1995). Microbiol. Rev. *59*, 143–159.

16. Torsvik, V., Ovreas, L., and Thingstad, T.F. (2002). Science *296*, 1064–1066.

17. Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., Wu, D., Paulsen, I., Nelson, K.E., Nelson, W., et al. (2004). Science *304*, 66–74.

18. Kehr, J.C., Zilliges, Y., Springer, A., Disney, M.D., Ratner, D.D., Bouchier, C., Seeberger, P.H., Tandeau de Marsac, N., and Dittmann, E. (2006). Mol. Microbiol. *59*, 893–906.